REMARKS

Claims 1 and 3-43 are pending. Of those claims, claims 3, 5, 9, 12-18, 21-22, 25-43 are withdrawn. Applicants have canceled claim 2 without prejudice or waiver of applicants' right to file for and obtain claims directed to any canceled subject matter in future divisional or continuing applications claiming priority from this application. Upon entry of this amendment, claims 1, 4, 6-8, 10, 11, 19, 20, 23 and 24 are under examination.

Applicants have amended claim 1 to specify that the cytokine-expressing cellular vaccine comprises proliferation-incompetent tumor cells that express GM-CSF. Support for this amendment is provided, for example, at specification page 4, lines 1; page 12, lines 13-22; page 15, lines 1-12; page 18, lines 10-11; page 20, lines 13-14 and lines 24-26; page 32, lines 1-4; and original claims 2 and 6.

Applicants have amended claims 4, 6-8, 11, 19, 23 and 24 to remove and correct the claim dependency from canceled claim 2.

None of the amendments introduces new matter.

THE REJECTIONS

35 U.S.C. § 112, first paragraph (enablement)
Claims 1, 2, 4, 6-8, 10, 11, 19, 20, 23, 24 and 26

The Examiner has rejected claims 1, 2 (canceled), 4, 6-8, 10, 11, 19, 20, 23, 24 and 26 (withdrawn) under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner contends that the specification does not provide a sufficient enabling description of a method for cancer therapy comprising administering a ***cellular vaccine*** in view of the Cancer Vaccine Fact Sheet from the National Cancer Institute (updated June

8, 2006). Specifically, the Examiner states that the Cancer Vaccine Fact Sheet states "that there are no licensed therapeutic vaccines to date" and concludes that it would take undue trial and error to practice the claimed invention. (Office Action, pp. 2-3).

Applicants have amended claim 1 (and claims dependent therefrom) to specify that the method for cancer therapy comprises administering a cytokine-expressing cellular vaccine comprising proliferation-incompetent tumor cells that express GM-CSF. The proliferation-incompetent tumor cells are administered in combination with at least one additional cancer therapeutic agent selected from a specific group of agents. Applicants respectfully submit that the specification provides adequate enablement for the claims as amended. The specification describes that vaccination of proliferation-incompetent (*e.g.*, by irradiation) tumor cells engineered to secrete GM-CSF stimulates potent, long-lasting and specific anti-tumor immunity that prevents tumor growth in a majority of mice challenged with non-transduced tumor cells (see, *e.g.*, p. 29, line 2 to p. 30, line 4; p. 31, lines 21-23; p. 39, line 28 to p. 40, lines 28-30; p. 40, lines 23-27). This is in contrast to the Examiner's contention that cellular vaccines would require undue experimentation.

The specification also describes that the combination of the cytokine-expressing cellular vaccine comprising proliferation-incompetent tumor cells that express GM-CSF and at least one additional cancer therapeutic agent is expected to increase the efficacy of anti-tumor protection. The specification describes several examples in which the combination of cellular vaccines comprising proliferation-incompetent tumor cells that express GM-CSF with cancer therapeutic agents results in enhanced therapeutic potency and efficacy relative to monotherapy (see, *e.g.*, Examples 1-8).

Based on these results, one of skill in the art would recognize that administering the specific combination of a cytokine-expressing cellular vaccine comprising proliferation-incompetent tumor cells that express GM-CSF with at least one additional cancer therapeutic agent selected from a specific group of agents, as recited in the amended claim, would result in enhanced therapeutic potency and/or efficacy relative to monotherapy. Thus, applicants respectfully submit that the present application provides sufficient enablement for one skilled in the art to make and use the invention without undue experimentation. Accordingly, applicants request that the Examiner withdraw the rejection.

35 U.S.C. § 112, first paragraph (written description)
Claim 1

The Examiner has rejected claim 1 under 35 U.S.C. § 112, first paragraph for lack of written description. The Examiner contends that applicants are not in possession of the term "cytokine-expressing cellular vaccine" because the specification discloses one example of a cytokine-expressing cellular vaccine, in particular, a vaccine expressing GM-CSF. The Examiner further contends that given the limited written description in the specification, the skilled artisan cannot envision all the contemplated structural possibilities of the cytokine-expressing cellular vaccine encompassed by the claims.

Applicants have amended claim 1 (and claims dependent therefrom) to specify that the cytokine-expressing cellular vaccine comprises proliferation-incompetent tumor cells that express GM-CSF, thereby obviating the Examiner's rejection. According, applicants request that the Examiner withdraw this rejection.

35 U.S.C. § 102(a)
Claims 1-2, 4, 6-7, 11, 19-20, 23-24 and 26

The Examiner has rejected claims 1, 2 (canceled), 4, 6-7, 11, 19-20, 23-24 and 26 (withdrawn) under 35 U.S.C. § 102(a) over Gri *et al.*, "OX40 ligand-transduced tumor cell vaccine synergizes with GM-CSF and requires CD40-Apc signaling to boost the host T cell antitumor response," *J. Immunol*, 170:99-106 (2003) ("*Gri*"). The Examiner states that *Gri* discloses a method of treating colon carcinoma by administering carcinoma cells transduced to express GM-CSF and OX40 ligand. The Examiner states that *Gri* teaches that OX40 ligand is functionally equivalent with anti-OX40 antibodies and concludes that a method of treating cancer by administering GM-CSF-expressing cells and anti-OX40 antibodies is inherent in the teachings of *Gri*. The Examiner further states that *Gri* further teaches that the GM-CSF-expressing cells are inactivated by irradiation and envisions using the method for human therapy.

Applicants respectfully traverse and submit that *Gri* fails to teach or suggest each and every limitation of the amended claims. In essence, the Examiner alleges that if OX-40 ligand can treat cancer when administered with GM-CSF-expressing cells (as suggested by *Gri*), then anti-OX40 antibodies must *necessarily* also treat cancer when administered with GM-CSF-expressing cells and, therefore, the claimed invention is inherently anticipated. But, for a reference to inherently anticipate, the unstated or implicit limitation or feature must "necessarily be present" when the reference is practiced or applied. In other words, the missing limitation must occur as a ***certainty***; the "mere *possibility*" that the reference may *sometimes* result in the limitation or characteristic is not enough. Here is how the Federal Circuit explained the inherency requirement in the well-known *Continental Can* case:

> Inherency, however, may not be established by probabilities or possibilities. **The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient.** If, however, the disclosure is sufficient to show that the natural result flowing from the operation as taught ***would result*** in the performance of the questioned function . . . the disclosure should be regarded as sufficient.
>
> *Continental Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1269 (Fed. Cir. 1991) (emphasis added).

In *Continental Can*, of course, the Federal Circuit vacated the district court's finding of inherent anticipation and remanded the case so the court could get it right. *See Schering Corp. v. Geneva Pharms. Inc.*, 339 F.3d 1373, 1377-1378 (Fed. Cir. 2003) (setting forth the "necessary and inevitable" test of inherent anticipation); *see also SmithKline Beecham Corp. v. Apotex Corp.*, 74 U.S.P.Q.2d 1398, 1407 (Fed. Cir. 2005) (accepting inherent anticipation argument because producing PHC anhydrate according to the earlier reference "inevitably results" in production of at least some of the claimed PHC hemihydrate).

Applicants respectfully submit that the *Gri* disclosure does not ***necessarily and inevitably*** lead to treatment of cancer when anti-OX40 antibodies are administered with GM-CSF-expressing cells. *Gri* discloses that only 30% of mice injected with C26 colon carcinoma cells transduced with both the GM-CSF gene and the OX-40 ligand gene (C26/GM/OX40L) developed tumors and half of the mice showed subsequent tumor regression. *Gri* also discloses that the overall survival of mice injected with C26/GM/OX40L was 85% and that all mice rejected a subsequent challenge with live C26 cells, indicating the development of immunological memory. *Gri* also discloses that when the dose of C26/GM/OX40L cells was increased, the tumor grew in all mice and

regressed in a smaller number of cells, suggesting that tumor burden is a major obstacle to immune-mediated rejection.

The Examiner points to the last paragraph of *Gri* on page 105 as his support that OX40 ligand is functionally equivalent to anti-OX40 antibodies. The Examiner's analysis is incorrect. The last paragraph of *Gri* states, in part, that "[r]ecent adoptive immunotherapy experiments have shown that the coadministration of anti-OX40 Ab reduces the number of transferred T cells required to obtain remission of pulmonary metastasis and intracranial tumors" (citing Kjaergaard *et al.*, "Therapeutic Efficacy of OX-40 Receptor Antibody Depends on Tumor Immunogenicity and Anatomic Site of Tumor Growth," Cancer Research, 60:5514-5521 (2000) ("*Kjaergaard*"), a copy of which is submitted herewith as **Exhibit A**).

To counter the Examiner's contention that the claims are inherently anticipated, Applicants point to the disclosure of *Kjaergaard* that describes experiments showing that anti-OX40 antibodies do not ***necessarily and inevitably*** treat cancers and that the therapeutic efficacy of anti-OX40 antibodies (referred to as OX40 receptor mAb in *Kjaergaard*) was influenced by a number of factors including the tumor burden, the intrinsic immunogenicity of the tumor as well as the histological site of tumor growth.

In particular, Applicants submit that *Kjaergaard* describes that "[w]hereas subdermal and intracranial growth of weakly immunogenic MCA 203 and MCA 205 sarcomas and GL261 glioma were susceptible to the mAb treatment, established ***pulmonary MCA 205 metastases were refractory to the same regimen of treatment.*** Furthermore, the mAb administration had ***no impact on the growth of the poorly immunogenic B16/D5 melanoma.***" (emphasis added; Abstract, p. 5514). *Kjaergaard* concludes that the "successful treatment is mAb dose-dependent and effected by the

intrinsic immunogenicity of tumors. It is also evident that the response of a particular tumor to the treatment varies and is dependent on the histological location of tumor growth." (see, p. 5517, first full paragraph). Based on *Kjaergaard*, anti-OX40 antibodies do not, in fact, ***necessarily and inevitably*** result in the treatment of cancer.

Thus, the simple relationship between OX-40 ligand and anti-OX40 antibodies that is presumed by the Examiner from the *Gri* disclosure is contradicted by the results described in *Kjaergaard*. That is, the effect of OX-40 ligand on the treatment of cancer would *not* have allowed one of skill in the art to predict the effect of anti-OX-40 antibodies on the treatment of cancer. Thus, there is not a necessary or inevitable correlation between the activities of OX-40 ligand and anti-OX40 antibodies.

Overall, Applicants submit that *Gri* fails to teach or suggest the claimed invention, either explicitly or inherently. Accordingly, Applicants submit that *Gri* fails to anticipate the instantly claimed methods and request that the rejection under 35 U.S.C. § 102(a) be withdrawn.

35 U.S.C. §§ 102(a) and (e)
Claims 1-2, 4, 6-8, 10, 11, 19-20, 23-24 and 26

The Examiner has rejected claims 1, 2 (canceled), 4, 6-8, 10, 11, 19-20, 23-24 and 26 (withdrawn) under 35 U.S.C. §§ 102(a) and 102(e) over US Patent Publication 2003/0035790 ("*Chen*"). The Examiner states that *Chen* discloses a method for treating cancer by administering a recombinant adenovirus engineered to express GM-CSF and an anti-OX-40 antibody. The Examiner states that *Chen* discloses that GM-CSF may be expressed in mammalian cells and discuss a cancer vaccine approach, wherein cancer cells are isolated from patients, transduced in vitro, irradiated, and administered to patients. The Examiner concludes that based on the disclosure in *Chen*, one of skill in the

art would immediately envisage a method wherein the GM-CSF expressing cells are administered, along with anti-OX-40 antibodies, to treat cancer. The Examiner further states that *Chen* discloses that the methods can be applied to treating prostate cancer.

Applicants have amended claim 1 (and claims dependent therefrom) to specify that the cytokine-expressing cellular vaccine comprises proliferation-incompetent tumor cells that express GM-CSF. Applicants respectfully submit that *Chen* fails to teach or suggest each and every limitation of the amended claims.

Chen discloses compositions and methods of treating diseases such as cancer by administering one or more compounds that activate one or more cytokine receptors and one or more compounds that activate one or more costimulatory molecules expressed by activated immune cells. In particular, *Chen* discloses injecting MCA26 tumor-bearing mice intratumorally with an adenovirus expressing mGM-CSF (see, *e.g.*, Example 10). *Chen* also discloses inducing metastatic colon cancer by implanting MCA26 tumor cells into the left lobe of the liver and then subsequently injecting adenovirus expressing mGM-CSF into tumor-bearing mice (see, *e.g.*, Example 11).

In contrast, the claimed invention relates to the administration of a cytokine-expressing cellular vaccine comprising proliferation-incompetent tumor cells that express GM-CSF. *Chen* does not teach or suggest this feature of the claimed invention. Rather than using proliferation-incompetent tumor cells to express GM-CSF, *Chen* describes injecting adenovirus expressing mGM-CSF directly into tumor-bearing animals. Therefore, *Chen* fails to teach or suggest this limitation of the amended claims.

Furthermore, the claimed invention also requires the administration at least one additional cancer therapeutic agent in combination with the cytokine-expressing cellular vaccine comprising proliferation-incompetent tumor cells that express GM-CSF,

wherein the combination results in an enhanced therapeutic effect compared to monotherapy. *Chen* also does not teach or suggest this particular combination. Therefore, *Chen* fails to teach or suggest each and every limitation of the amended claims. Accordingly, applicants request that the Examiner withdraw this rejection.

35 USC §101 - Nonstatutory Double Patenting
Claims 1-2, 4, 6-8, 10, 11, 19-20, 23-24 and 26

The Examiner has provisionally rejected claims 1, 2 (canceled), 4, 6-8, 10, 11, 19-20, 23-24 and 26 (withdrawn) on the ground of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 1-33 of copending Application No. 10/404,662.

Applicants request that the present basis for the provisional rejection be held in abeyance until applicants are notified that claims in the instant application are otherwise allowable.

35 U.S.C. § 103(a) – Provisional rejection
Claims 1-2, 4, 6-8, 10, 11, 19-20, 23-24 and 26

The Examiner states that claims 1, 2 (canceled), 4, 6-8, 10, 11, 19-20, 23-24 and 26 (withdrawn) are directed to an invention not patentably distinct from claim 1-33 of commonly assigned Application No. 10/404,662. The Examiner states that the USPTO normally will not institute an interference between applications or a patent and an application of common ownership (citing MPEP Chapter 2300). The Examiner states that commonly assigned Application No. 10/404,662 would form the basis for a rejection of the noted claims under 35 U.S.C. § 103(a) if the commonly assigned case qualifies as prior art under 35 U.S.C. § 102(e), (f) or (g) and the conflicting inventions were not commonly owned at the time the invention in this application was made. The Examiner

states that in order to resolve this issue, the assignee can, under 35 U.S.C. § 103(c) and 35 C.F.R. § 1.78(c), either show that the conflicting inventions were commonly owned at the time the invention in this application was made, or name the prior inventor of the conflicting subject matter.

Applicants respectfully submit that the invention of copending Application No. 10/404,662 and the present claimed invention were commonly owned by and subject to assignment to Cell Genesys, Inc. at the time the present claimed invention was made. The inventors of copending Application No. 10/404,662 (Moskalenko, Li, Aung, Prell, Creson, and Jooss) were all employed by and obligated to assign inventions to Cell Genesys, Inc. at the time the subject matter in Application No. 10/404,662 was invented. The inventors of the pending case (Moskalenko, Li, Aung, Prell, Creson, Jooss and Du) were all employed by and obligated to assign inventions to Cell Genesys, Inc. at the time the subject matter in the pending case was invented. *See* the assignment for Application No. 10/404,662 (Reel 014451, Frame 0187) (executed August 8, 2003 and August 19, 2003) **(Exhibit B)** and the assignment in the pending case (Reel 015754, Frame 0515) (executed July 1, 2004) **(Exhibit C)** (copies enclosed). Applicants also enclose copies of the *Notice of Recordation of Assignment* as received from the USPTO for Application No. 10/404,662 (**Exhibit D**, recorded September 2, 2003) and the pending case (**Exhibit E**, recorded September 2, 2004). Applicants respectfully submit that they have satisfied the requirements under 35 U.S.C. § 103(c) and 35 C.F.R. § 1.78(c) to show that the conflicting inventions were commonly owned at the time the invention in this application was made.

CONCLUSION

In view of the above, applicants request that the Examiner examine the pending claims in this application. Applicants request favorable consideration and early allowance of the pending claims.

Respectfully submitted,

Connie Wong

James F. Haley, Jr. (Reg. No. 27,794)
Connie Wong (Reg. No. L0104)
Attorneys for Applicants
c/o ROPES & GRAY LLP
(Customer No. 1473)
1211 Avenue of the Americas
New York, New York 10036
Telephone (212) 596-9000

Applicants: Karin Jooss et al.
Application No.: 10/807,449
Express Mail Label No. EM015927105US

Attorney Docket No.: 105576-0033-102
Confirmation No.: 3354

Exhibit A (8 pages)

[CANCER RESEARCH 60, 5514–5521, October 1, 2000]

Therapeutic Efficacy of OX-40 Receptor Antibody Depends on Tumor Immunogenicity and Anatomic Site of Tumor Growth[1]

Jørgen Kjærgaard, Junta Tanaka, Julian A. Kim, Kevin Rothchild, Andrew Weinberg, and Suyu Shu[2]

Center for Surgery Research-FF50, The Cleveland Clinic Foundation, Cleveland, Ohio 44195 [J. K., J. T., J. A. K., K. R., S. S.], and Providence Portland Medical Center, Earle A. Chiles Research Institute, Portland, Oregon 97213 [A. W.]

ABSTRACT

The OX-40 receptor (OX-40R) is a cell surface glycoprotein of the tumor necrosis factor receptor family that is expressed primarily on activated CD4 T cells. Engagement of OX-40R by the OX-40 ligand (OX-40L) is known to costimulate the production of cytokines by activated T lymphocytes and to rescue effector T cells from activation-induced cell death. It was previously reported that *in vivo* ligation of OX-40R by administration of OX-40L:immunoglobulin fusion protein or OX-40R monoclonal antibody (mAb) resulted in a significant prolongation of survival of tumor-bearing mice in four histologically distinct solid tumors. In this study, we demonstrate that the therapeutic efficacy of OX-40R mAb was influenced by the tumor burden, the intrinsic immunogenicity of the tumor as well as by the histological site of tumor growth. Whereas subdermal and intracranial growth of weakly immunogenic MCA 203 and MCA 205 sarcomas and GL261 glioma were susceptible to the mAb treatment, established pulmonary MCA 205 metastases were refractory to the same regimen of treatment. Furthermore, the mAb administration had no impact on the growth of the poorly immunogenic B16/D5 melanoma. Tumor regression mediated by OX-40R mAb was dependent on the participation of both CD4 and CD8 T cells and as a result of tumor rejection, a long-term tumor-specific immunity was established. Analysis of tumor-infiltrating T cells revealed the presence of a far greater number of OX-40R$^+$ T cells of both CD4 and CD8 phenotypes in the intracranial immunogenic GL261 glioma than that in the poorly immunogenic B16/D5 melanoma. These results suggest that ligation of OX-40R on activated T cells *in situ* in the tumor may provide a necessary costimulatory signal to augment immune responses leading to tumor regression and immunological memory.

INTRODUCTION

An important goal of cancer immunology and immunotherapy is to understand fundamental principles of immune responses to tumors. This will lead to the design of strategies to enhance tumor antigen recognition by cells of the immune system and to channel reactive pathways for therapeutic benefits. Rejection of tumors through immune responses is primarily mediated by T cells. Growing evidence indicates that T cells require at least two physiologically distinct signals to become activated (1, 2). The first signal is generated by the interaction of T cell antigen receptors and antigenic peptide/MHC class I or II complexes on APCs[3], *e.g.*, tumor cells or dendritic cells. The second signal is delivered by costimulation molecules on APCs through their counter receptors on T cells (3–6). Without costimulation, exposure of T cells to antigen may induce unresponsiveness or anergy (7, 8). Thus, augmentation of costimulation has been an attractive approach to enhance weak immune responses during progressive tumor growth.

There are several known molecules that can provide costimulation (5, 9). The best characterized costimulatory signal is the one delivered to the T-cell CD28 receptor by its ligand B7.1 (CD80) or B7.2 (CD86). In many murine tumors, transduction of tumor cells to express B7.1 or B7.2 resulted in the loss of tumorigenicity (10–12). Immunization with such modified tumor cells elicited protective immunity against challenges with wild-type tumors and in some cases, mediated regression of existing tumors (10). In addition, several other membrane-bound receptor-ligand pairs can also serve to be costimulators for T-cell activation. In particular, members of the tumor necrosis factor receptor superfamily have been shown to share the ability to enhance or costimulate the process of T-cell activation (13–17). This family consists of the CD30, CD40, CD27, Fas (CD95), DR3, 4–1BB, and OX-40. The 4–1BB receptor binds to a high-affinity ligand (4–1BBL) expressed on several APCs such as dendritic cells, macrophages, and activated B cells. Expression of 4–1BB is somewhat restricted to primed CD4, CD8 T cells, and natural killer cells (18). Of particular significance is the observation that administration of 4–1BB mAb as a single agent could eradicate well-established tumors in mice (19). Although both CD4 and CD8 participated in the antitumor immune responses, the stimulation of a CD8 CTL response was particularly striking. The CTL activity generated from 4–1BB mAb-treated mice was increased up to approximately 65 times compared with that of spleen cells from control animals. Thus, ligation of costimulation receptors *in vivo* may augment natural immunity to the growing tumors sufficient to induce their regression.

Until recently, the OX-40 receptor-ligand costimulation system has received relatively little attention for exploitation of it ability to enhance antitumor immunity because due to the fact that the receptor expression was reportedly confined to primed CD4 T cells only (20). The OX-40R has a very distinct pattern of expression in animals with EAE. At the inflammatory site, it appears that T cells expressing the OX-40R are cells that recognized the autoantigen and were involved in the pathogenesis of EAE (21–23). Additional analyses of tumor-infiltrating lymphocytes and tumor-draining lymph nodes from melanoma, breast cancer, and head and neck cancer patients identified the presence of OX-40R$^+$ cells (24, 25). These findings along with our recent demonstration that tumor-specific CD4 T cells isolated from L-selectine^{low} cell population of tumor-draining lymph nodes mediated tumor regression without the participation of CD8 T cells (26) have inspired the inception of the hypothesis that *in vivo* ligation of the OX-40R on T cells may augment of antitumor immunity.

Recent work has tested the therapeutic efficacy of the OX-40L: immunoglobulin fusion protein as well as specific OX-40R mAb for the treatment of four antigenically and histologically distinct murine tumors (25). In each tumor, treatment by ligation of OX-40R *in vivo* resulted in a significant improvement in survival of the tumor-bearing mice. The anti-OX-40R effects were dose-dependent and immunologically mediated. In the current study, we further analyzed the immunomodulatory function of the *in vivo* administered OX-40R mAb in mice bearing tumors of various immunogenicities as well as tumors inoculated at different anatomical sites. Our results suggest that the

Received 3/24/00; accepted 8/3/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by USPHS Grants RO1-CA81383, RO1-CA78263, and R37-CA67324 from the National Cancer Institute, and by National Cancer Institute Grant R01-CA81383-01 and Cantab Pharmaceuticals grant (to A. W.).

[2] To whom requests for reprints should be addressed, at Center for Surgery Research-FF50, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195. Phone: (216) 445-3800; Fax: (216) 445-3805; E-mail: shus@ccf.org.

[3] The abbreviations used are: APC, antigen-presenting cell; mAb, mononuclear antibody; EAE, experimental autoimmune encephalomyelitis; OX-40R, OX-40 receptor; OX-40L, OX-40 ligand; CM, complete medium; s.d., subdermal/subdermally; BBB, blood-brain barrier; IL, interleukin.

therapeutic responses may be predicated to the extent of OX-40R$^+$ T lymphocytes infiltrating the growing tumor.

MATERIALS AND METHODS

Animals. Female C57BL/6N (B6) mice, 6–8 weeks old, were purchased from the Biological Testing Branch, Frederick Cancer Research and Development Center, National Cancer Institute (Frederick, MD). They were maintained in a specific pathogen-free environment according to National Institutes of Health guidelines and were used for experiments at the age of 8–12 weeks.

Tumors. The MCA 203 and MCA 205 fibrosarcomas are 3-methylcholanthrene-induced tumors of B6 origin (27). The tumors have been routinely passed *in vivo* by serial s.c. transplantation in syngeneic mice and were used within the fifth to the eight transplantation generation. Single-cell suspensions were prepared from solid tumors by digestion with a mixture of 0.1% collagenase, 0.01% DNase, and 2.5 units/ml hyaluronidase (Sigma, St. Louis, MO) for 3 h at room temperature. The cells were filtered through a layer of no. 100 nylon mesh, washed, and resuspended in HBSS. B16/D5 is a poorly immunogenic subclone of the spontaneously arising B16/BL6 melanoma (28). The B16/D5 tumor does not exhibit a detectable level of MHC class I (H-2 D^b and K^b) and class II molecules. These tumor cells were maintained in culture in CM. CM consisted of RPMI 1640 supplemented with 10% heat-inactivated FCS, 0.1 mM nonessential amino acids, 1 μM sodium pyruvate, 2 mM fresh L-glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin, 50 μg/ml gentamicin, and 0.5 μg/ml fungizone (all from Life Technologies, Grand Island, NY), and 5×10^{-5} M 2-mercaptoethanol (Sigma). GL261 glioma, originally induced by intracranial implantation of methylcholanthrene pellets in a B6 mouse, was obtained from the Division of Cancer Treatment Repository (Frederick, MD; 29). The GL261 tumor cells was maintained in continuous culture in CM. Cultured B16/D5 or GL261 tumor cells were harvested after a short incubation at 37°C with a solution containing 0.25% trypsin and 0.02% EDTA (Life Technologies, Inc., Grand Island, NY). The tumor cells were washed and resuspended in HBSS for animal inoculation.

Tumor Inoculation. B6 mice were given s.d. injections with 1×10^5 to 1.5×10^6 MCA 205 tumor cells suspended in 100 μl of HBSS to initiate tumor growth. The diameters of s.d. tumors were measured twice weekly with a Vernier caliper, and size was recorded as an average of perpendicular measurements and presented as individual tumor growth curves. Mice were killed when the dermal tumor reached a size greater than 20 mm. To establish intracranial tumors, B6 mice were anesthetized with 0.8 mg of pentobarbital i.p. and inoculated with 10 μl of tumor cell suspension transcranially using a 27-gauge needle and glass tuberculin syringe (Perfectum; Popper & Sons, Inc, New Hyde Park, NY). The needle insertion was perpendicular to the skull and in line with the anterior margin of the ear and the medial half of the right eye. The depth of insertion was controlled by placement of electric wire insulation as a collar over the needle with exposure of the terminal 4 mm. To establish pulmonary metastases, mice were given i.v. injections with 1×10^5 MCA 205 tumor cells suspended in 1 ml of HBSS. On day 21, mice were killed, and metastatic tumor nodules on the surface of the lung were enumerated after counterstaining with India ink.

mAb to OX-40R and Treatment Protocol. Hybridoma that produced the antimurine OX-40R-specific mAb (termed OX-86) was obtained from the European Collection of Animal Cell Cultures (30). The hybridoma was grown in RPMI medium containing 10% FCS that was depleted of immunoglobulin by passing it over protein G columns. The cells were grown to high density, and the supernatant was poured over a protein G column, and purified antibody was eluted. Antibody concentrations were quantitated by absorption at 280 nm. In most experiments, tumor-bearing mice were treated with the mAb on days 3 and 7 after tumor inoculation by i.p. injections. Doses of mAb are indicated in the figure legends.

Depletion of T-Cell Subsets *in Vivo*. Ascites for the rat IgG2b mAb against murine CD4 (GK1.5, L3T4) and CD8 (2.43, Lyt 2.2) was produced in sublethally irradiated (500 R), cyclophosphamide (2 mg/mouse)-treated DBA/2 mice. Mice with 3-day established intracranial GL261 tumors were depleted of T-cell subsets by i.v. injection of 0.2 ml of ascites fluid diluted to 1.0 ml with HBSS prior to OX-40R mAb treatment. T cell depletion was confirmed by flow cytometric analyses of spleen cells from treated animals.

Isolation and Characterization of T Cells from Intracranial Tumors and Lymphoid Organs. Anesthetized B6 mice with 10-day established GL261 or B16/D5 intracranial tumors were perfused through the left ventricle of the heart with 3 ml of HBSS before removal of the brain. Single-cell suspensions were prepared by digesting minced brain tissue in 40 ml of HBSS containing 10 mg collagenase (type IV, Sigma) for 60 min at room temperature. The cell suspensions were washed in HBSS, resuspended in 10 ml of 50% Percoll (Pharmacia, Uppsala, Sweden), and then overlaid with 4 ml of 30% Percoll to form a discontinuous gradient in a 15-ml centrifuge tube. The gradient was centrifuged at 4°C for 40 min at $1000 \times g$. Cells recovered from the interphase were washed in HBSS before flow cytometric analysis. Single-cell suspensions from lymph nodes or spleens were prepared mechanically by teasing organs with needles followed by pressing tissue fragments with the blunt end of a plastic syringe. Cells were stained by indirect immunofluorescence for the expression of OX-40R using OX40L:immunoglobulin fusion protein and FITC-conjugated antihuman IgG (Caltag, South San Francisco, CA). Cells were also stained with PE-conjugated anti-CD4 or CD8. The membrane fluorescence was analyzed using FACSCalibur (Becton Dickinson, Sunnyvale, CA).

Statistical Analysis. The significance of differences in numbers of pulmonary metastases between groups was analyzed by the Wilcoxon rank-sum test. Differences of numbers of cells infiltrating tumor tissues were analyzed by the Student's t test. A two-tailed P of ≤ 0.05 was considered significant.

RESULTS

Tumor Rejection Induced by OX-40R Antibody Administration. In a recent report, the therapeutic effects of *in vivo* administration of either OX-40L:immunoglobulin fusion protein or OX-40R-specific mAb in four histologically distinct murine tumors have been demonstrated (25). The effects appear to be affected by the intrinsic immunogenicity of the experimental tumor. In the present study, we further extended these observations by examining the therapeutic effects of OX-40R mAb in different animals models and in situations in which tumors were growing at different anatomical sites. We initially treated mice bearing s.d. tumors of the weakly immunogenic MCA 205 fibrosarcoma with OX-40R mAb (150 μg i.p.) on days 3 and 7. Fig. 1 depicts the results in which mice inoculated with 1×10^5 (Fig. 1, *Expt. 1*) or 3×10^5 (Fig. 1, *Expt. 2*) MCA 205 tumor cells responded to the treatment resulting in complete tumor eradication in 3 of 5 animals. In mice inoculated with 1.5×10^6 tumor cells (Fig. 1, *Expt. 3*), treatment with OX-40R mAb resulted in some retardation and delay of tumor growth, but all eventually succumbed to the progressive tumors. In all of the tumor doses, inoculation led to progressive s.d. tumor growth in control mice. These results. thus, indicate that tumor burden may be a limiting factor to the therapeutic response of the OX-40R mAb treatment.

Therapeutic Efficacy of OX-40R mAb for the Treatment of Intracranial Tumors. Although the brain has long been considered to be an immunologically privileged site, our extensive studies have demonstrated the successful treatment of experimental intracranial tumors by the systemic transfer of activated tumor-specific immune T cells (26, 31). We, therefore, examined the therapeutic effects of OX-40R mAb against intracranial tumors. Of the three weakly immunogenic tumors, MCA 205, MCA 203 sarcomas, and GL261 glioma, intracerebral inoculations of 1×10^5 tumor cells consistently resulted in progressive growth of tumors in the brain and killed the host with a median survival time of approximately 20 days. Mice bearing MCA 205 intracranial tumors responded to the treatment with OX-40R mAb and demonstrated the efficacy of therapy was antibody dose-dependent. Treatment with 150 μg mAb i.p. on days 3, 7, and 11 prolonged the survival, but only one of six treated animals was cured (Fig. 2*A*). Treatment with higher antibody doses, either by four administrations of 150 μg on days 3, 7, 11, and 15 or by two administrations of 300 μg on days 3 and 7, demonstrated a higher

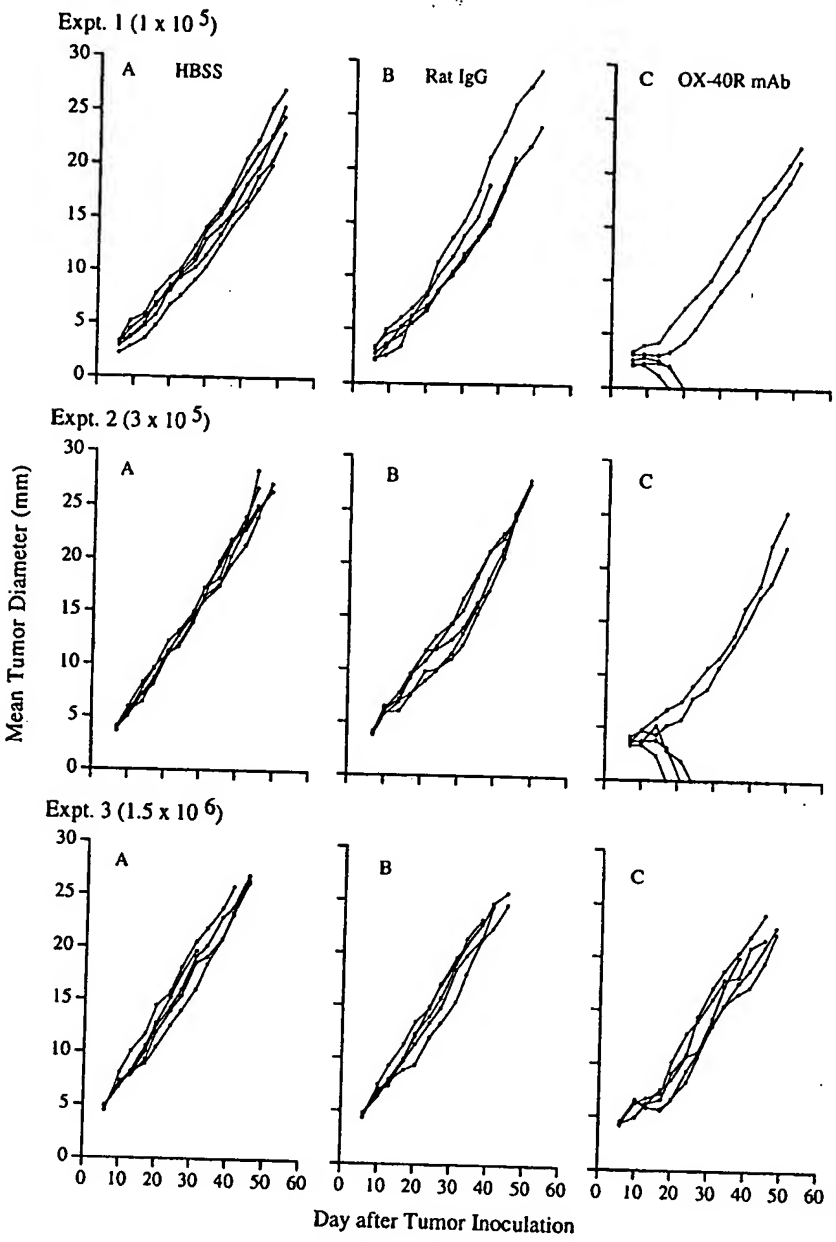

Fig. 1. Induction of antitumor immunity by OX-40R mAb against s.d. tumors. B6 mice in groups of five were inoculated s.d. with 1×10^5 (*Expt. 1*), 3×10^5 (*Expt. 2*), or 1.5×10^6 (*Expt. 3*) MCA 205 tumor cells. On days 3 and 7, mice were treated by i.p. injections of HBSS (*A*), control rat IgG (150 μg/injection; *B*), or OX-40R mAb (150 μg/injection; *C*). Tumor sizes were estimated by measuring perpendicular diameters, and the results are expressed as mean diameters of tumors in individual mice.

therapeutic efficacy resulting in complete eradication of tumors in 50% of the treated mice. Similarly, for the treatment of MCA 203 sarcoma and GL261 glioma, i.p. injection of mice with 300 μg of OX-40R mAb on days 3 and 7 prolonged survival, and 3 and 4 of 6 treated animals were cured of tumors, respectively (Fig. 2, *B* and *C*). Increasing the dose of GL261 tumor cells in the tumor inoculum resulted in decreased therapeutic effects of the antibody treatment (Fig. 2*D*). In an additional experiment, mice inoculated intracerebrally with 10^5 MCA 205 tumor cells were treated with two injections of 300 μg of OX-40R mAb 4 days apart commencing on day 3, 5, or 7. Whereas 2 of 5 mice treated beginning on day 3 were cured, all of the mice succumbed to the progressive growing tumors when treatment was delayed (data not shown). These results thus confirm that the tumor burden is a limiting factor as seen in previously experiments (Fig. 1).

In an attempt to establish a model system for the treatment of poorly immunogenic tumors, we used a cloned tumor cell line, B16/D5, which was derived from the B16/F10/BL6 melanoma. Previous experimental results suggested that the B16/D5 tumor failed to immunize syngeneic animals using irradiated tumor cells or admixed with *Corynebacterium parvum* as an adjuvant. In addition, lymph nodes draining the B16/D5 tumor contained very few immune effector cells when adoptively transferred to treat 3-day established pulmonary metastases (32). In the present study, intracerebral inoculation of as few as 1×10^3 tumor cells led to progressive tumor growth that eventually killed the animals, with a median survival time of 18 days. Treatment of intracranial B16/D5 tumor-bearing mice with two i.p. administration of 300 μg OX-40R mAb on days 3 and 7 did not demonstrate any therapeutic effects (Fig. 2*E*).

A similar strategy was used to treat pulmonary metastases derived from the MCA 205 sarcoma. Mice were injected i.v. with 1×10^5 tumor cells suspended in 1.0 ml of HBSS to establish multiple metastases in the lung. Such a model system has been used extensively in the past for evaluating therapeutic efficacy of both active and adoptive immunotherapies. Mice bearing pulmonary metastases were treated exactly as described above with two i.p. injections of 300 μg of OX-40R mAb on days 3 and 7. On day 21, all of the mice were killed and metastatic nodules on the surface of the lung were esti-

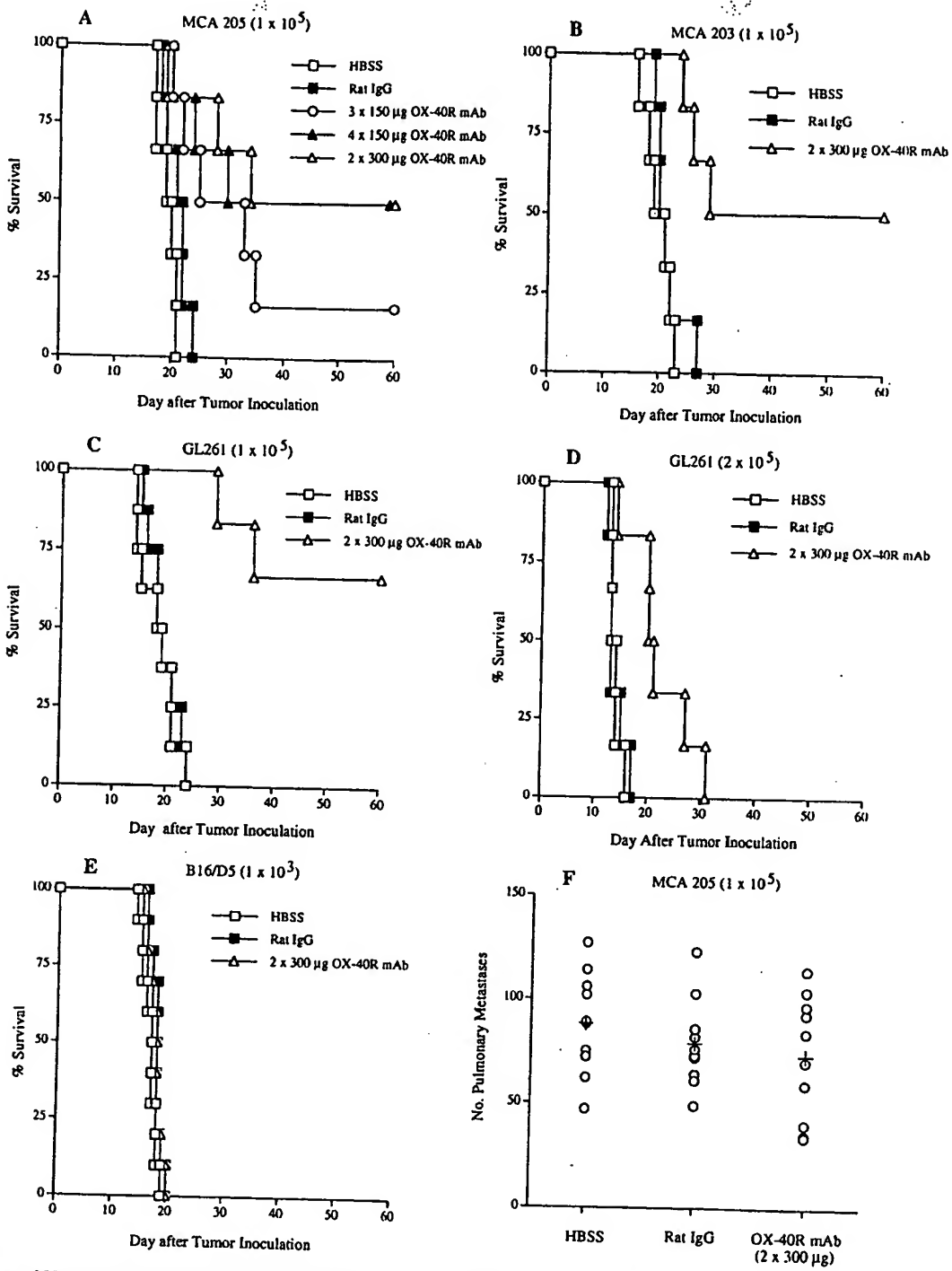

Fig. 2. Antitumor effects of OX-40R mAb administration against intracranial tumors. In *A*, B6 mice in groups of six were inoculated intracranially (i.c.) with 1×10^5 MCA 205 tumor cells. They were treated by multiple i.p. injections of HBSS on days 3, 7, 11, and 15 (□), rat IgG (■, 300 μg/injection on days 3 and 7), or OX-40R mAb (○, 150 μg/injection on days 3, 7, and 11; ▲, 150 μg/injection on days 3, 7, 11, and 15; △, 300 μg/injection on days 3 and 7). In *B*, B6 mice in groups of six were inoculated i.c. with 1×10^5 MCA 203 tumor cells. On days 3 and 7, mice were treated by i.p. injections of HBSS, rat IgG (300 μg/injection), or OX-40R mAb (300 μg/injection). In *C* and *D*, B6 mice in groups of six were inoculated i.c. with 1×10^5 (*C*) or 2×10^5 (*D*) GL261 glioma cells. On days 3 and 7, mice received i.p. injections of HBSS, rat IgG (300 μg/injection), or OX-40R mAb (300 μg/injection). In *E*, B6 mice in groups of 10 were inoculated i.c. with 1×10^3 B16/D5 melanoma cells. On days 3 and 7, mice were treated by i.p. injections of HBSS, rat IgG (300 μg/injection), or OX-40R mAb (300 μg/injection). In *F*, B6 mice in groups of five were inoculated i.v. with 1×10^5 MCA 205 tumor cells to establish pulmonary metastases. On days 3 and 7, mice were treated by i.p. injections of HBSS, rat IgG (300 μg/injection), or OX-40R mAb (300 μg/injection). Compilations of two independent experiments because of similar results.

mated. Despite its effectiveness for the treatment of s.d. and intracranial MCA 205 tumors, the antibody failed to effect tumor growth as judged by the numbers of metastatic nodules in treated mice as compared with controls (Fig. 2*F*).

Cumulatively, this series of experiments confirms the therapeutic effectiveness of OX-40R ligation in the induction of tumor eradication as claimed previously (25). The successful treatment is mAb dose-dependent and effected by the intrinsic immunogenicity of tumors. It is also evident that the response of a particular tumor to the treatment varies and is dependent on the histological location of tumor growth.

Specificity and Long-Term Immunity after Successful Treatment. To determine whether a long-lasting immunity was induced in mice that were cured by the OX-40R mAb treatment, we challenged survival animals, 60 days after initial tumor inoculation, with either

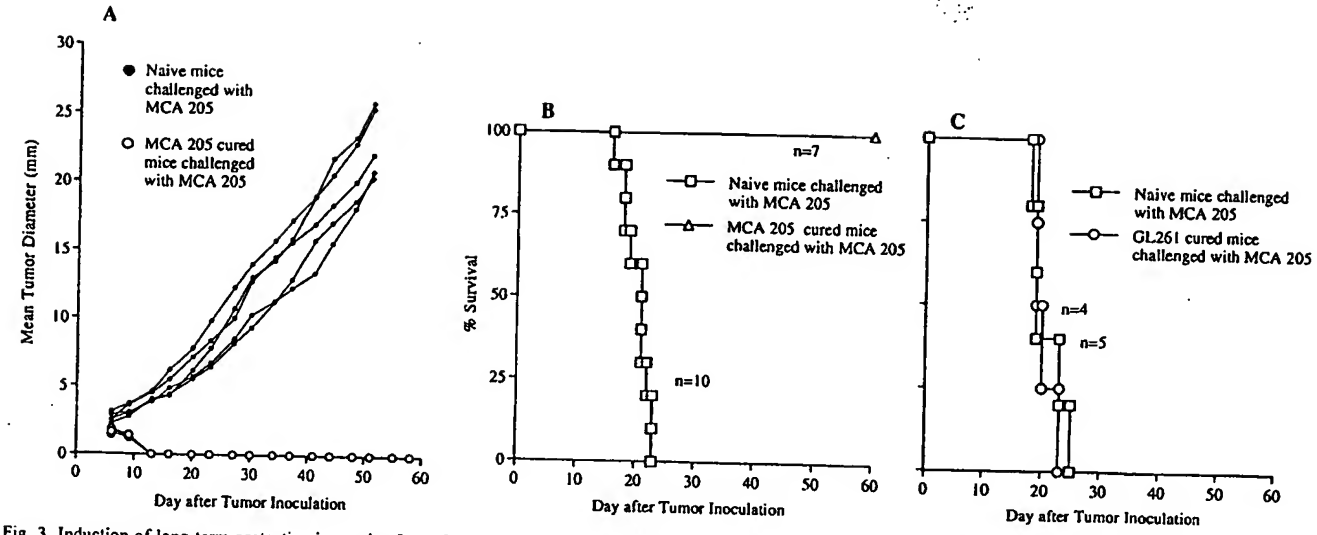

Fig. 3. Induction of long-term protective immunity. In *A*, four B6 mice that had been cured of the s.d. MCA 205 tumor, and remained tumor-free for 60 days, were rechallenged s.d. with 3×10^5 MCA 205 tumor cells (○). After a period of 8 days transient growth, all of the tumor nodules regressed, as compared with progressive tumor growth in all of the control normal mice (●). In *B*, the seven mice from the Fig. 2*A* experiment that had been cured of intracranial (i.c.) MCA 205 tumor, and remained tumor-free for 60 days, were rechallenged i.c. with 1×10^5 MCA 205 (△). Naive normal mice were similarly challenged (□). In *C*, the four mice from the Fig. 2C experiment that had rejected the i.c. GL261 tumor, and remained tumor-free for 60 days, were challenged i.c. with 1×10^5 MCA 205 (○). Naive normal mice served as control (□).

the same tumor or an antigenically distinct tumor. All of the mice cured of MCA 205 primary s.d. tumors (see Fig. 1) resisted a s.d. rechallenge with 3×10^5 MCA 205 tumor cells, whereas control, naive mice showed progressive tumor growth after tumor challenge (Fig. 3*A*). Similarly, mice that survived the intracranial MCA 205 tumors were resistant to a second intracranial challenge (1×10^5) with the same tumor (Fig. 3*B*). We also tested the specificity of this long-term immunity by rechallenging the mice that survived the intracranial GL261 tumor as a consequence of OX-40R mAb treatment with the antigenically distinct MCA 205 tumor. As can be seen in Fig. 3*C*, the growth of MCA 205 intracranial tumors has not been affected by the initial rejection of the GL261 tumor. These results indicate that the tumor eradication triggered by OX-40R mAb established a long-lasting tumor-specific immunological memory.

Role of T-Cell Subsets in OX-40R mAb-induced Antitumor Response. The above experiments strongly suggest that tumor eradication by OX-40R mAb treatment was an indirect result of the augmentation of antitumor immune responses. Because specificity and long-term immunity are maintained by T cells, we examined the role of different T-cell subsets in OX-40R mAb-mediated tumor regression. Mice inoculated intracranially with GL261 tumor cells were treated with two courses of OX-40R mAb (300 μg/each) i.p. on days 3 and 7. Cohorts of treated mice were also injected i.v. with CD4 (GK1.5) or CD8 (2.43) mAbs to deplete corresponding T-cell subsets on day 3 prior to the first OX-40R mAb treatment. The method of *in vivo* depletion of T-cell subsets with mAb has been a routine procedure in our laboratory. Flow cytometric analysis of spleen cells 14 days after mAb T-cell depletion revealed a reduction from ~20% in untreated mice to <2% of CD4 or CD8 T cells in the treated mice. In the experiment depicted in Fig. 4, mice treated with OX-40R mAb without T-cell depletion showed prolongation of survival and three of six mice were cured of the tumor. In animals depleted of either CD4 or CD8 T cells, the antitumor effects of the OX-40R mAb were abrogated. Although OX-40R has been primarily expressed on activated CD4 T cells, and the GL261 glioma does not express MHC class II molecules, our results suggest that tumor regression induced by the OX-40R mAb treatment required the participation of both CD4 and CD8 host T cells.

Expression of OX-40R on Tumor-infiltrating T Lymphocytes and the Susceptibility of the Tumor to mAb Treatment. Successful treatment of tumors by the OX-40R ligation with mAb seemed to be influenced by the intrinsic immunogenicity of the target tumor (see Fig. 2). One possible mechanism responsible for the tumor susceptibility is the abundance of tumor-sensitized precursor T cells during the growth of immunogenic tumors. We, therefore, attempted to define the OX-40R expression on tumor-associated T cells isolated from 10-day-old tumors of both the susceptible immunogenic GL261 glioma and the resistant poorly immunogenic B16/D5 melanoma. Because successful OX-40R mAb treatment resulted in tumor-specific systemic immunity, we also examined the expression of OX-40R on splenic T cells from tumor-bearing mice. Quantitatively, in the intracranial GL261 tumor, there were ~25% tumor-associated T cells, which is 6-fold more T cells than could be detected in the B16/D5 tumor (~4.5%, Table 1). It is noteworthy that among tumor-infiltrating T cells, there were 26 ± 3% of the GL261 tumor-associated CD4

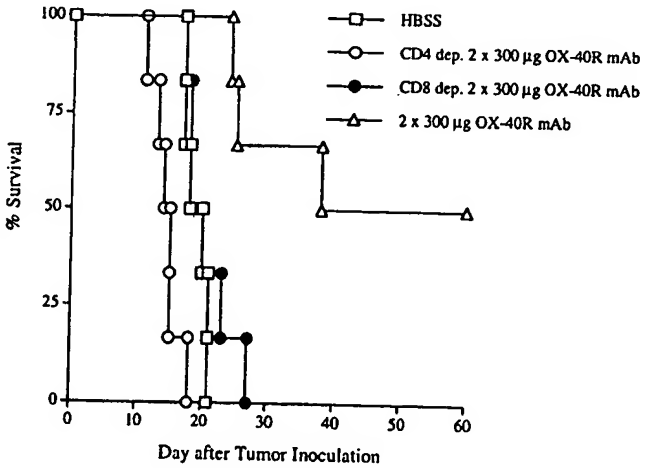

Fig. 4. Role of CD4 and CD8 T cells in OX-40R mAb induced intracranial (i.c.) tumor regression. B6 mice with 3-day GL261 i.c. glioma were depleted of CD4 or CD8 T cells by i.v. injections of GK1.5 (○) or 2.43 (●) mAb, respectively, on day 3, 2 h before i.p. treatment with OX-40R mAb (300 μg/injection). The mice were treated again by i.p. administration of OX-40R mAb on day 7 (300 μg/injection).

Table 1 *Contents of OX-40R$^+$ T cells in tumor mass*[a]

Tumor	% T cells in tumor mass		% OX-40R expression on	
	CD4	CD8	CD4	CD8
GL261	13.6 ± 2.5	11.1 ± 1.3	26.3 ± 3.4	10.7 ± 3.0
B16/D5	2.8 ± 1.0	1.7 ± 1.0	11.3 ± 3.3	3.9 ± 1.0

[a] Average of three independent experiments.

cells expressing OX-40R, whereas only 11 ± 3% of OX-40R$^+$ CD4 cells were detected in the B16/D5 tumor (Fig. 5). Unexpectedly, 11 ± 3% and 4 ± 1% of CD8 cells isolated from GL261 and B16/D5 tumors were OX-40R$^+$, respectively. Analysis of OX-40R expression on splenic T cells of GL261-bearing mice revealed that ~15% of CD4 cells expressed OX-40R, whereas only ~7% of CD4 T cells from B16/D5-bearing mice were OX-40R$^+$. In both tumor systems, no OX-40R$^+$ CD8 T cells were detected in the spleen (Fig. 5) or lymph nodes (data not shown). It is possible that OX-40R$^+$ T cells in the tumors are the candidate for targeting by the mAb. The observations that the therapeutic efficacy is dependent on both the CD4 and CD8 T cells and the immunogenicity of the treated tumor, support this possibility.

DISCUSSION

The OX-40R has been reported to be expressed on T cells isolated from the inflammatory site in several inflammatory diseases, including rheumatoid arthritis, graft-*versus*-host disease, and EAE, which suggests that signaling through OX-40R may be involved in modulating immune reactions (21–23, 33). In murine models, blockade of the OX-40R signaling has been described to ameliorate the pathogenesis of EAE (34) and hapten-induced colitis (35), and, more recently, to attenuate alloantigen-specific CTL response (36). These observations underscore the role of OX-40R signaling in the regulation of immune responses. However, very little information is available with regard to its role in the immune response to malignancies. This may largely reflect the restriction in the expression of OX-40R, which seemed to confine to CD4 T lymphocytes (20), and the well-documented, dominant role of CD8 CTL in the immune response to tumors. It was not until recently that CD4 T cells alone were demonstrated to mediate the regression of established tumors (26).

The results of current studies have extended previous findings that *in vivo* engagement of OX-40R by administration of OX-40L:immunoglobulin fusion proteins or OX-40R mAb resulted in significant therapeutic benefits in four histologically and immunologically distinct murine tumors (25). Whereas our results emphasized the critical role of the immunogenicity of tumors to the effects of OX-40R mAb treatment, the histological location of tumor growth was also a determining factor affecting the outcome of treatment. It is clear that s.d. and intracranial tumors were susceptible to the antibody treatment in a dose-dependent manner. Established pulmonary metastases from the same tumor seemed to be refractory to the effects of mAb. To explain the difficulty in treating metastases in the lung, it is possible that micrometastases may not facilitate sufficient intratumoral infiltration of activated T cells for mAb targeting as compared with solitary s.d. and intracranial tumors. This is supported by our recent observation, in which therapy with the adoptive transfer of tumor-specific T cells in conjunction with OX-40R mAb administration significantly accelerated the antitumor immunity against 10-day established pulmonary

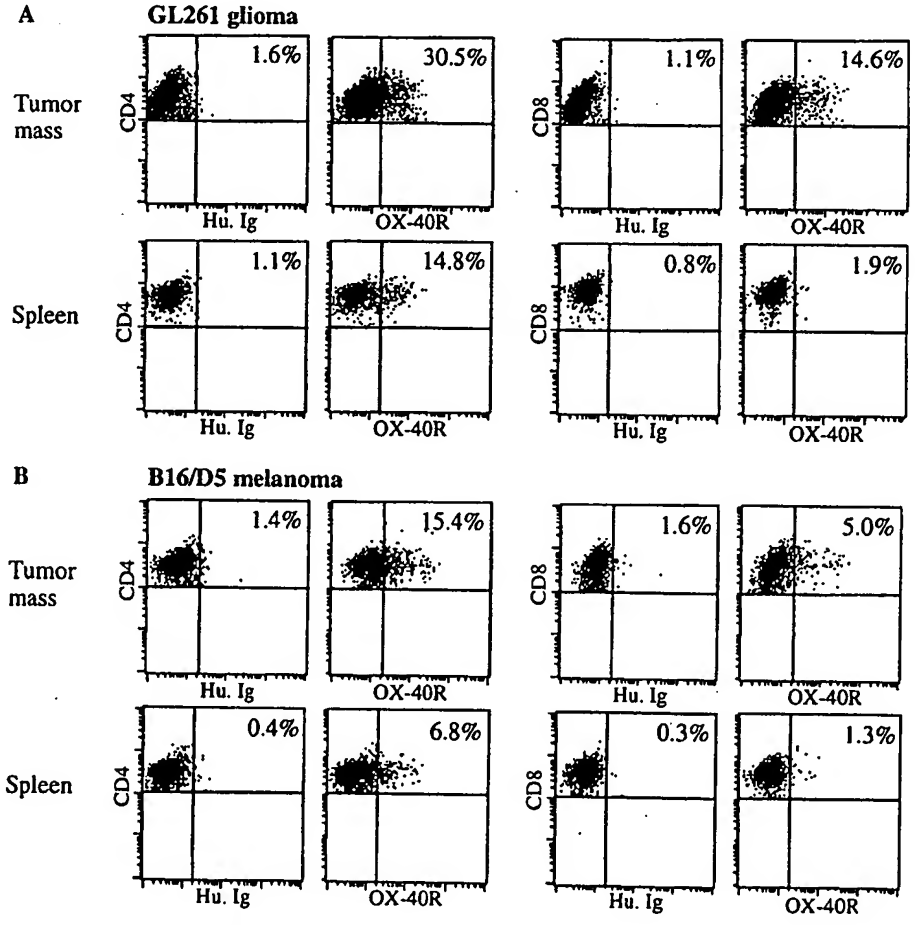

Fig. 5. Expression of OX-40R on T lymphocytes isolated from intracranial GL261 glioma or B16/D5 melanoma. Mononuclear cell suspensions were isolated and stained with murine OX-40L:Fc-immunoglobulin chimeric protein followed with FITC-conjugated anti-human IgG, before being double-stained with either PE-conjugated anti-CD4 or -CD8. By two-color flow cytometry, samples were gated on CD4 or CD8 T cells to display the expression of OX-40R on each subset. *Upper right quadrants*, the proportions (%) of the CD4 or CD8 T cells expressing OX-40R are indicated. Data are representative of three experiments.

metastases.[4] It is, therefore, possible that advanced pulmonary metastases might be more susceptible to the treatment effects of mAb. This hypothesis is being tested experimentally.

The responsiveness of intracranial tumors to the systemic OX-40R mAb treatment is intriguing because the brain is considered to be an immunologically privileged site because of the existence of the BBB. It is also however, possible that the growing tumor in the brain disrupts the integrity of the BBB, which allows the entry of antibodies. Because the OX-40R mAb does not directly interact with tumor cells, and its function is targeting the activated T cells by ligation of OX-40R on their surface (23), an initial immune response must have occurred to generate activated T cells that express the OX-40R. Although inflammatory cells are rare in the normal brain, increased leukocyte entry occurs in pathological conditions. The physical BBB using tight endothelial junctions and glial end-feed, blocks passive entry of large molecules but does not stop the active entry of living cells (37, 38). Furthermore, in the normal brain, MHC expression is minimal. However, both MHC class I and II expression can be up-regulated on microglia and endothelial cells under the influence of IFN-γ and other cytokines (39, 40). These cells may serve the function of APCs; thus, the growing brain tumors may have created a microenvironment conducive to initiating an immune response. Indeed, not only have we observed the expression of OX-40R on the tumor-associated or tumor-infiltrating lymphocytes but the total number of cells and percentages of OX-40R positive cells seems to predict their responses to the OX-40R mAb treatment.

We are aware that the intracerebral route of injection may have damaged the BBB, perhaps permitting local entry of lymphocytes and the mAb. However, this is unlikely to occur solely after a local injury, because there is a lack of inflammatory response in the brain after intracerebral injection of sterile PBS (41). The progressive growth of a tumor, on the other hand, may disrupt the integrity of the BBB even in naturally occurring tumors. It has been reported that the primary human gliomas are often infiltrated with lymphocytes, and in the peripheral circulation, there exists lymphocytes specifically reactive to glioma cells (42, 43). In fact, survival of the glioma patients correlates with the degree of lymphocytic infiltration in the tumor. Nevertheless, we cannot entirely exclude minor injury as a contributing factor in the success of antibody-mediated tumor regression.

The finding that the poorly immunogenic B16/D5 melanoma was not responsive to the therapeutic effects of OX-40R mAb has several explanations. It is possible that B16/D5 tumor cells either lack molecules that can serve sufficiently as tumor antigens recognized by T cells or are deficient in the processing, transportation or presentation of such molecules by APCs. It is, relevant therefore, to note that MHC molecules (both class I and II) are not detectable on B16/D5 cells. In many poorly immunogenic tumors, increasing MHC class I expression by transfection with MHC class I genes or IFN-γ cDNA resulted in an enhanced sensitivity to CTL lysis *in vitro* and an increased infiltration of tumor by CD8 lymphocytes *in vivo* (10, 44). Furthermore, B16 melanoma cells transduced with a gene encoding granulocyte-macrophage colony-stimulating factor could elicit a protective immunity against challenges with wild-type tumor cells (45). This finding may be explained by the production of cytokines and/or enhanced expression of MHC and costimulatory molecules induced by granulocyte-macrophage colony-stimulating factor. Taken together, the failure to treat the B16/D5 melanoma may be primarily attributable to insufficient triggering of the initial immune response. If this is the case, transduction of tumor cells to express MHC molecules or IFN-γ may provide a means of increasing responsiveness to the OX-40R mAb treatment against poorly immunogenic tumors.

As discussed earlier, the administration of 4–1BB mAb eradicates established tumors in mice (19). Analysis of mechanisms of tumor rejection revealed the participation of both CD4 and CD8 in tumor-bearing mice. Because the 4–1BB glycoprotein is expressed on both of the activated CD4 and CD8 T cells (18), it is logical to hypothesize that the antibody amplifies both CD4 and CD8 immune responses. In contrast to the 4–1BB, the OX-40R has been repeatedly demonstrated to be preferentially expressed by activated CD4 T cells (20, 23, 24). Our recent studies have demonstrated that appropriately sensitized and activated CD4 T cells alone could mediate potent antitumor responses when adoptively transferred to tumor-bearing mice (26). Because of these findings, we hypothesized a restricted role of OX-40R mAb to be amplifying CD4 T cell-mediated antitumor reactivity. However, T cell phenotype analysis indicated that both CD4 and CD8 T cells were required for effective treatment with the OX-40R mAb. It was first interpreted that OX-40R signaling on CD4 T cells provided an increased T-helper function, which in turn facilitated a CD8 CTL response. To support this hypothesis is the observation that the adoptive transfer of CD8-depleted spleen cells from OX-40L:immunoglobulin cured mice conferred resistance to tumor challenge in naive mice (25). Because of the demonstration of OX-40R$^+$ CD8 T cells in tumor-infiltrating lymphocytes, it is also possible that the mAb directly binds to CD8 T cells and activates them. CD8 T cells have the potential to express the OX-40R after stimulation with potent mitogens such as ConA and PHA (46).

The mechanism by which administered OX-40R mAb may assist the generation of antitumor responses or enhance existing antitumor immunity is poorly understood. It has previously been shown that sensitized CD4 T cells exposed to Ag and cocultured with B7.1/OX-40L expressing fibroblasts, demonstrate enhanced proliferation, IL-2 secretion, and prolonged survival (47). Thus, one possibility is that the tumor-infiltrating T lymphocytes, sensitized *in vivo* to tumor antigens, were at a stage of differentiation that permitted proliferation and activation by the OX-40R mAb ligation. Because most antigen-sensitized T cells become susceptible to activation-induced cell death, and only a minority differentiate to become memory T cells (48), ligation of the OX-40R on antigen-activated T cells may abrogate or delay the impact of activation-induced cell death leading to increased Ag-specific memory (49). In addition, OX-40R mAb treatment may shift the balance between Th1 and Th2 immune responses. Blocking of OX-40R signaling has resulted in reducing *in vivo* transcript for Th1 cytokines such as tumor necrosis factor-α, IFN-γ, IL-2 and IL-12 in animals with EAE and bowel disease (34, 35). However, in studies with naive CD4 T cells, *in vitro* activation by the OX-40L resulted in promoting Th2 cell development (50, 51). Shifting of type 1/type 2 T cell functions as a possible mechanism of OX-40R mAb-mediated tumor regression remains to be determined.

In summary, ligation of OX-40R *in vivo* with antibodies can lead to delaying of tumor progression and, in some cases, eradicating tumors. The demonstration of OX-40R$^+$ T cells in tumor-infiltrating lymphocytes suggests that they are the targets of antibody binding and activation. Therapeutic efficacy of the antibody, however, seemed to be limited by the tumor burden. Recently, it has been demonstrated that immune responses are regulated, at least in part, by naturally occurring CD4$^+$, CD25$^+$ T cells in normal animal (52). In some animal tumor model systems, depletion of this population of regulatory cells resulted in enhanced immune responses and tumor eradication (53, 54). Because the therapeutic effect of the OX-40R mAb is indirect through the induction of antitumor immune responses, it may

[4] Unpublished observation.

be possible to improve the therapeutic efficacy by the removal of $CD4^+$, $CD25^+$ T cells. This hypothesis is being tested in our laboratory. Finally, the significance of this approach is underscored by the fact that similar $OX\text{-}40R^+$ T cells have been demonstrated in several human malignancies including melanoma, head and neck carcinoma, and carcinoma of the breast (24, 25). If these cells prove to be tumor-sensitized T lymphocytes, ligation of OX-40R mAb may provide tumor Ag-specific therapeutic benefits in cancer patients.

REFERENCES

1. Bretscher, P. The two-signal model of lymphocyte activation twenty-one years later. Immunol. Today, *13:* 74–76, 1992.
2. Mueller, D. L., Jenkins, M. K., and Schwartz, R. H. Clonal expansion *versus* functional clonal inactivation: a costimulatory signaling pathway determines the outcome of T cell antigen receptor occupancy. Annu. Rev. Immunol., *7:* 445–480, 1989.
3. Guinan, E. C., Gribben, J. G., Boussiotis, V. A., Freeman, G. J., and Nadler, L. M. Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. Blood, *84:* 3261–3282, 1994.
4. Bluestone, J. A., and Lechler, R. I. Transplantation. Curr. Opin. Immunol., *7:* 617–619, 1995.
5. Lenschow, D. J., Walunas, T. L., and Bluestone, J. A. CD28/B7 system of T cell costimulation. Annu. Rev. Immunol., *14:* 233–258, 1996.
6. Lenschow, D. J., Herold, K. C., Rhee, L., Patel, B., Koons, A., Qin, H. Y., Fuchs, E., Singh, B., Thompson, C. B., and Bluestone, J. A. CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes. Immunity, *5:* 285–293, 1996.
7. Kearney, E. R., Pape, K. A., Loh, D. Y., and Jenkins, M. K. Visualization of peptide-specific T cell immunity and peripheral tolerance induction *in vivo*. Immunity, *1:* 327–339, 1994.
8. Croft, M., Joseph, S. B., and Miner, K. T. Partial activation of naive CD4 T cells and tolerance induction in response to peptide presented by resting B cells. J. Immunol., *159:* 3257–3265, 1997.
9. Croft, M., and Dubey, C. Accessory molecule and costimulation requirements for CD4 T cell response. Crit. Rev. Immunol., *17:* 89–118, 1997.
10. Tanaka, K., Gorelik, E., Watanabe, M., Hozumi, N., and Jay, G. Rejection of B16 melanoma induced by expression of a transfected major histocompatibility complex class I gene. Mol. Cell. Biol., *8:* 1857–1861, 1988.
11. Chen, L., McGowan, P., Ashe, S., Johnston, J. V., Hellstrom, J., and Hellstrom, K. E. B7-1/CD80-transduced tumor cells elicit better systemic immunity than wild-type tumor cells admixed with *Corynebacterium parvum*. Cancer Rec., *54:* 5420–5423, 1994.
12. Joki, T., Kikuchi, T., Akasaki, Y., Saitoh, S., Abe, T., and Ohno, T. Induction of effective antitumor immunity in a mouse brain tumor model using B7-1 (CD80) and intercellular adhesive molecule 1 (ICAM-1; CD54) transfection and recombinant interleukin 12. Int. J. Cancer., *82:* 714–720, 1999.
13. Durie, F. H., Foy, T. M., Masters, S. R., Laman, J. D., and Noelle, R. J. The role of CD40 in the regulation of humoral and cell-mediated immunity. Immunol. Today, *15:* 406–411, 1994.
14. Grewal, I. S., and Flavell, R. A. The role of CD40 ligand in costimulation and T-cell activation. Immunol. Rev., *153:* 85–106, 1996.
15. Shuford, W. W., Klussman, K., Tritcher, D. D., Loo, D. T., Chalupny, J., Siadak, A. W., Brown, T. J., Emswiler, J., Raecho, H., Larsen, C. P., Pearson, T. C., Ledbetter, J. A., Aruffo, A., and Mittler, R. S. 4–1BB costimulatory signals preferentially induce $CD8^+$ T cell proliferation and lead to the amplification *in vivo* of cytotoxic T cell responses. J. Exp. Med., *186:* 47–55, 1997.
16. Miyawaki, T., Uehara, T., Nibu, R., Tsuji, T., Yachie, A., Yonehara, S., and Taniguchi, N. Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood. J. Immunol., *149:* 3753–3758, 1992.
17. Watanabe-Fukunaga, R., Brannan, C. I., Copeland, N. G., Jenkins, N. A., and Nagata, S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature (Lond.), *356:* 314–317, 1992.
18. Vinay, D. S., and Kwon, B. S. Role of 4–1BB in immune responses. Sem. Immunol., *10:* 481–489, 1998.
19. Melero, I., Shuford, W. W., Newby, S. A., Aruffo, A., Ledbetter, J. A., Hellstrom, K. E., Mittler, R. S., and Chen, L. Monoclonal antibodies against the 4–1BB T-cell activation molecule eradicate established tumors. Nat. Med., *3:* 682–685, 1997.
20. Paterson, D. J., Jefferies, W. A., Green, J. R., Brandon, M. R., Corthesy, P., Puklavec, M., and Williams, A. F. Antigens of activated rat T lymphocytes including a molecule of 50,000 Mr detected only on CD4 positive T blasts. Mol. Immunol., *24:* 1281–1290, 1987.
21. Weinberg, A. D., Lemon, M., Jones, A. J., Vainiene, M., Celnik, B., Buenafe, A. C., Culbertson, N., Bakke, A., Vandenbark, A. A., and Offner, H. OX-40 antibody enhances for autoantigen specific $V_\beta 8.2^+$ T cells within the spinal cord of Lewis rats with autoimmune encephalomyelitis. J. Neurosci. Res., *43:* 42–49, 1996.
22. Buenafe, A. C., Weinberg, A. D., Culbertson, N. E., Vandenbark, A. A., and Offner, H. Vβ CDR3 motifs associated with BP recognition are enriched in $OX\text{-}40^+$ spinal cord T cells of Lewis rats with EAE. J. Neurosci. Res., *44:* 562–567, 1996.
23. Weinberg, A. D., Bourdette, D. N., Sullivan, T. J., Lemon, M., Wallin, J. J., Maziarz, R., Davey, M., Palida, F., Godfrey, W., Engleman, E., Fulton, R. J., Offner, H., and Vandenbark, A. A. Selective depletion of myelin-reactive T cells with the anti-OX-40 antibody ameliorates autoimmune encephalomyelitis. Nat. Med., *2:* 183–189, 1996.
24. Vetto, J. T., Lum, S., Morris, A., Sicotte, M., Davis, J., Lemon, M., and Weinberg, A. D. Presence of the T-cell activation marker OX-40 on tumor infiltrating lymphocytes and draining lymph node cells from patients with melanoma and head and neck cancers. Am. J. Surg., *174:* 258–265, 1997.
25. Weinberg, A. D., Rivera, M. M., Prell, R., Morris, A., Ramstad, T., Vetto, J. T., Urba, W. J., Alvord, G., Bunce, C., and Shields, J. Engagement of the OX-40 receptor *in vivo* enhances antitumor immunity. J. Immunol., *164:* 2160–2169, 2000.
26. Kagamu, H., and Shu, S. Purification of $L\text{-selectin}^{low}$ cells promotes the generation of highly potent CD4 antitumor effector T lymphocytes. J. Immunol., *160:* 3444–3452, 1998.
27. Shu, S., and Rosenberg, S. A. Adoptive immunotherapy of newly induced murine sarcomas. Cancer Res., *45:* 1657–1662, 1985.
28. Hart, I. R. The selection and characterization of an invasive variant of the B16 melanoma. Am. J. Pathol., *97:* 587–600, 1979.
29. Ausman, J. I., Shapiro, W. R., and Rall, D. P. Studies of the chemotherapy of experimental brain tumors: development of an experimental model. Cancer Res., *30:* 2394–2400, 1970.
30. al-Shamkhani, A., Birkeland, M. L., Puklavec, M., Brown, M. H., James, W., and Barclay, A. N. OX-40 is differentially expressed on activated rat and mouse T cells and is the sole receptor for the OX-40 ligand. Eur. J. Immunol., *26:* 1695–1699, 1996.
31. Inoue, M., Plautz, G. E., and Shu, S. Treatment of intracranial tumors by systemic transfer of superantigen-activated tumor-draining lymph node T cells. Cancer Res., *56:* 4702–4708, 1996.
32. Hu, H. M., Urba, W. J., and Fox, B. A. Gene-modified tumor vaccine with therapeutic potential shifts tumor-specific T cell response from a type 2 to a type 1 cytokine profile. J. Immunol., *161:* 3033–3041, 1998.
33. Tittle, T. V., Weinberg, A. D., Steinkeler, C. N., and Maziarz, R. T. Expression of the T cell activation antigen, OX-40, identifies alloreactive T cells in acute graft-*versus*-host disease. Blood, *89:* 4652–4658, 1997.
34. Weinberg, A. D., Wegmann, K. W., Funatake, C., and Whitham, R. H. Blocking OX-40/OX-40 ligand interaction *in vitro* and *in vivo* leads to decreased T cell function and amelioration of experimental allergic encephalomyelitis. J. Immunol., *162:* 1818–1826, 1999.
35. Higgins, L. M., McDonald, S. A., Whittle, N., Crockett, N., Shields, J. G., and MacDonald, T. T. Regulation of T cell activation *in vitro* and *in vivo* by targeting the OX40-OX40 ligand interaction: amelioration of ongoing inflammatory bowel disease with an OX40-IgG fusion protein, but not with an OX40 ligand-IgG fusion protein. J. Immunol., *162:* 486–493, 1999.
36. Murata, K., Ishii, N., Takano, H., Miura, S., Ndhlovu, L. C., Nose, M., Noda, T., Sugamura, K. Impairment of antigen-presenting cell function in mice lacking expression of OX40 ligand. J. Exp. Med., *191:* 365–374, 2000.
37. Knobler, R. L., Marini, J. C., Goldowitz, D., and Lublin, F. D. Distribution of the blood-brain barrier in heterotopic brain transplants and its relationship to the lesions of EAE. J. Neuropath. Exp. Neurol., *51:* 36–39, 1992.
38. Andersson, P. B., Perry, V. H., and Gordon, S. The acute inflammatory response to lipopolysaccharide in CNS parenchyma differs from that in other body tissues. Neuroscience, *48:* 169–186, 1992.
39. Dhib-Jalbut, S., Kufta, C. V., Flerlage, M., Shimojo, N., and McFarland, H. F. Adult human glial cells can present target antigens to HLA-restricted cytotoxic T-cells. J. Neuroimmunol., *29:* 203–211, 1990.
40. Balabanov, R., Beaumont, T., and Dore-Duffy, P. Role of central nervous system microvascular pericytes in activation of antigen-primed splenic T-lymphocytes. J. Neurosc. Res., *55:* 578–587, 1999.
41. Mitchell, M. S., Kempf, R. A., Harel, W., Shau, H., Boswell, W. D., Lind, S., and Bradley, E. C. Effectiveness and tolerability of low-dose cyclophosphamide and low-dose intravenous interleukin-2 in disseminated melanoma. J. Clin. Oncol., *6:* 409–424, 1988.
42. Brooks, W. H., and Roszman, T. L. Cellular immune responsiveness of patients with primary intracranial tumors. *In:* C. D. G. T. Thomas and D. I. Graham (eds.), Brain Tumors, Scientific Basis, Clinical Investigation and Current Therapy, pp. 121–132. London: Butterworths, 1980.
43. Palma, L., DiLorenzo, N, and Guidetti, B. Lymphocytic infiltrates in primary glioblastomas and recidivous gliomas. Incidence, fate, and relevance to prognosis in 228 operated cases. J. Neurosurg., *49:* 854–861, 1978.
44. Restifo, N. P., Spiess, P. J., Karp, S. E., Mule, J. J., and Rosenberg, S. A. A nonimmunogenic sarcoma transduced with cDNA for interferon gamma elicits $CD8^+$ T cells against the wild-type tumor: correlation with antigen presentation capability. J. Exp. Med., *175:* 1423–1431, 1992.
45. Yu, J. S., Burwick, J. A., Dranoff, G., and Breakefield, X. O. Gene therapy for metastatic brain tumors by vaccination with granulocyte-macrophage colony-stimulating factor-transduced tumor cells. Hum. Gene Ther., *8:* 1065–1072, 1997.
46. Baum, P. R., Gayle, R. B., Ramsdell, F., Srinivasan, S., Sorensen, R. A., Watson, M. L., Seldin, M. F., Baker, E., Sutherland, G. R., Clifford, K. N., Alderson, M. R., Goodwin, R. G., and Fanslow, W. C. Molecular characterization of murine and human OX-40/OX-40 ligand systems: identification of a human OX-40 ligand as the HTLV-1-regulated protein gp34. EMBO J., *13:* 3992–4001, 1994.
47. Gramaglia, I., Weinberg, A. D., Lemon, M., and Croft, M. OX-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. J. Immunol., *161:* 6510–6517, 1998.
48. Zhang, X., Brunner, T., Carter, L., Dutton, R. W., Rogers, P., Bradley, L., Sato, T., Reed, J. C., Green, D., and Swain, S. L. Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. J. Exp. Med., *185:* 1837–1849, 1997.
49. Maxwell, J. R., Weinberg, A., Prell, R. A., and Vella, T. Danger and OX40 receptor signaling synergize to enhance memory T cell survival by inhibiting peripheral deletion. J. Immunol., *164:* 107–112, 2000.
50. Ohshima, Y., Yang, L. P., Uchiyama, T., Tanaka, Y., Baum, P., Sergeric, M., Hermann, P., and Delespesse, G. OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human $CD4^+$ T cells into high IL-4-producing effectors. Blood, *92:* 3338–3345, 1998.
51. Flynn, S., Toellner, K. M., Raykundalia, C., Goodall, M., and Lane, P. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulate expression of the chemokine receptor, Blr-1. J. Exp. Med., *188:* 297–304, 1998.
52. Suri-Payre, E., Amar, A. Z., Thornton, A. M., and Shevach, E. M. $CD4^+CD25^+$ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J. Immunol., *160:* 1212–1218, 1998.
53. Onizuka, S., Tawara, J., Shimizu, J., Sakaguchi, S., Fujita, T., and Nakayama, E. Tumor rejection by *in vivo* administration of anti-CD25 (interleukin-2 receptor α) monoclonal antibody. Cancer Res., *59:* 3128–3133, 1999.
54. Shimizu, J., Yamazaki, S., and Sakaguchi, S. Induction of tumor immunity by removing $CD25^+CD4^+$ T cells: a common basis between tumor immunity and autoimmunity. J. Immunol., *163:* 5211–5218, 1999.

Applicants: Karin Jooss et al.
Application No.: 10/807,449
Express Mail Label No. EM015927105US

Attorney Docket No.: 105576-0033-102
Confirmation No.: 3354

Exhibit B (7 pages)

ASSIGNMENT

For good and valuable consideration, the receipt of which is hereby acknowledged, the person(s) named below (referred to as "INVENTOR" whether singular or plural) has sold, assigned, and transferred and does hereby sell, assign, and transfer to **Cell Genesys, Inc.** a **Delaware** corporation, having a place of business at **500 Forbes Blvd., South San Francisco, California 94080**, ("ASSIGNEE"), for itself and its successors, transferees, and assignees, the following:

1. The entire worldwide right, title, and interest in all inventions and improvements ("SUBJECT MATTER") that are disclosed in the provisional application filed under 35 U.S.C. §111(b) or non-provisional application filed under 35 U.S.C. §111(a) and entitled CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS ("APPLICATION"), which:

☐ is to be filed herewith

☒ was filed on April 2, 2003
now bearing U.S. application number 10/404,662; and

2. The entire worldwide right, title, and interest in and to:

(a) the APPLICATION; (b) all applications claiming priority from the APPLICATION; (c) all utility, divisional, continuation, substitute, renewal, reissue, and other related applications thereto which have been or may be filed in the United States or elsewhere in the world; (d) all patents (including reissues and re-examinations) which may be granted on the applications set forth in (a), (b), (c) and (d) above; and (e) all right of priority in the APPLICATION, together with all rights to recover damages for infringement of provisional rights.

INVENTOR agrees that ASSIGNEE may apply for and receive patents for SUBJECT MATTER in ASSIGNEE's own name.

INVENTOR agrees to do the following, when requested, and without further consideration, in order to carry out the intent of this Assignment: (1) execute all oaths, assignments, powers of attorney, applications, and other papers necessary or desirable to fully secure to ASSIGNEE the rights, titles and interests herein conveyed; (2) communicate to ASSIGNEE all known facts relating to the SUBJECT MATTER; and (3) generally do all lawful acts that ASSIGNEE shall consider desirable for securing, maintaining, and enforcing worldwide patent protection relating to the SUBJECT MATTER and for vesting in ASSIGNEE the rights, titles, and interests herein conveyed. INVENTOR further agrees to provide any successor, assign, or legal representative of ASSIGNEE with the benefits and assistance provided to ASSIGNEE hereunder.

INVENTOR represents that INVENTOR has the rights, titles, and interests to convey as set forth herein, and covenants with ASSIGNEE that the INVENTOR has not made and will not hereafter make any assignment, grant, mortgage, license, or other agreement affecting the rights, titles, and interests herein conveyed.

INVENTOR grants the attorney of record the power to insert on this Assignment any further identification that may be necessary or desirable in order to comply with the rules of the United States Patent and Trademark Office for recordation of this document.

This Assignment may be executed in one or more counterparts, each of which shall be deemed an original and all of which may be taken together as one and the same Assignment.

Title of Document: **ASSIGNMENT**

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) April 2, 2003
Application No.: (if applicable) 10/404,662

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Marina Moskalenko	8/8, 2003	8/8, 2003

State of CALIFORNIA }S.S.
County of SAN MATEO }

On August 8, 2003 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared MARINA MOSKALENKO

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Notary Seal

Title of Document: **ASSIGNMENT**

Re:
Title: **CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS**
Filed: (if applicable) April 2, 2003
Application No.: (if applicable) 10/404,662

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Betty Li	8/8/03, 2003	8/8/03, 2003

State of CALIFORNIA }S.S.
County of SAN MATEO }

On August 8, 2003 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared BETTY LI

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Notary Seal

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) April 2, 2003
Application No.: (if applicable) 10/404,662

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Sandra Aung	08/08, 2003	08/08, 2003

State of CALIFORNIA }S.S.
County of SAN MATEO }

On August 8, 2003 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared SANDRA AUNG

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Notary Seal

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) April 2, 2003
Application No.: (if applicable) 10/404,662

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Rodney Prell	8/8/, 2003	8/8/, 2003

State of CALIFORNIA }S.S.
County of SAN MATEO }

On August 8, 2003 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared RODNEY PRELL

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that he executed the same in his authorized capacity and that by his signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Notary Seal

Notary

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) April 2, 2003
Application No.: (if applicable) 10/404,662

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Jennifer Creson Jennifer Creson	Aug 19, 2003	Aug 19, 2003

State of CALIFORNIA }S.S.
County of SAN MATEO }

On August 19, 2003 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared JENNIFER CRESON

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Notary Seal

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) April 2, 2003
Application No.: (if applicable) 10/404,662

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Karin Jooss	8-8-, 2003	8-8, 2003

State of CALIFORNIA }S.S.
County of SAN MATEO }

On August 8, 2003 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared KARIN JOOSS

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Notary Seal

Applicants: Karin Jooss et al.
Application No.: 10/807,449
Express Mail Label No. EM015927105US

Attorney Docket No.: 105576-0033-102
Confirmation No.: 3354

Exhibit C (8 pages)

ASSIGNMENT

For good and valuable consideration, the receipt of which is hereby acknowledged, the person(s) named below (referred to as "INVENTOR" whether singular or plural) has sold, assigned, and transferred and does hereby sell, assign, and transfer to Cell Genesys, Inc. a Delaware corporation, having a place of business at 500 Forbes Blvd., South San Francisco, California 94080, ("ASSIGNEE"), for itself and its successors, transferees, and assignees, the following:

1. The entire worldwide right, title, and interest in all inventions and improvements ("SUBJECT MATTER") that are disclosed in the provisional application filed under 35 U.S.C. §111(b) or non-provisional application filed under 35 U.S.C. §111(a) and entitled CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS ("APPLICATION"), which:

☐ is to be filed herewith

☒ was filed on March 24, 2004
now bearing U.S. application number 10/807,449; and

2. The entire worldwide right, title, and interest in and to:

(a) the APPLICATION; (b) all applications claiming priority from the APPLICATION; (c) all utility, divisional, continuation, substitute, renewal, reissue, and other related applications thereto which have been or may be filed in the United States or elsewhere in the world; (d) all patents (including reissues and re-examinations) which may be granted on the applications set forth in (a), (b), (c) and (d) above; and (e) all right of priority in the APPLICATION, together with all rights to recover damages for infringement of provisional rights.

INVENTOR agrees that ASSIGNEE may apply for and receive patents for SUBJECT MATTER in ASSIGNEE's own name.

INVENTOR agrees to do the following, when requested, and without further consideration, in order to carry out the intent of this Assignment: (1) execute all oaths, assignments, powers of attorney, applications, and other papers necessary or desirable to fully secure to ASSIGNEE the rights, titles and interests herein conveyed; (2) communicate to ASSIGNEE all known facts relating to the SUBJECT MATTER; and (3) generally do all lawful acts that ASSIGNEE shall consider desirable for securing, maintaining, and enforcing worldwide patent protection relating to the SUBJECT MATTER and for vesting in ASSIGNEE the rights, titles, and interests herein conveyed. INVENTOR further agrees to provide any successor, assign, or legal representative of ASSIGNEE with the benefits and assistance provided to ASSIGNEE hereunder.

INVENTOR represents that INVENTOR has the rights, titles, and interests to convey as set forth herein, and covenants with ASSIGNEE that the INVENTOR has not made and will not hereafter make any assignment, grant, mortgage, license, or other agreement affecting the rights, titles, and interests herein conveyed.

INVENTOR grants the attorney of record the power to insert on this Assignment any further identification that may be necessary or desirable in order to comply with the rules of the United States Patent and Trademark Office for recordation of this document.

This Assignment may be executed in one or more counterparts, each of which shall be deemed an original and all of which may be taken together as one and the same Assignment.

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) March 24, 2002
Application No.: (if applicable) 10/807,449

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Karin Jooss	7-1, 2004	, 2004

State of CALIFORNIA }S.S.
County of SAN MATEO }

On July 01, 2004 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared KARIN U. JOOS

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

Notary Seal

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) March 24, 2002
Application No.: (if applicable) 10/807,449

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Jennifer Creson	July 1, 2004	, 2004

Jennifer Creson

State of CALIFORNIA }S.S.
County of SAN MATEO }

On July 01, 2004 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared JENNIFER R. CRESON

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

Notary Seal

MGC1 ANDREW P. SALVADOR COMM. # 1349346 NOTARY PUBLIC-CALIFORNIA SAN MATEO COUNTY COMM. EXP. APRIL 29, 2006 MGC1

Andrew P. Salvador
Notary

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) March 24, 2004
Application No.: (if applicable) 10/807,449

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Betty Li	7/1/04, 2004	, 2004

State of CALIFORNIA }S.S.
County of SAN MATEO }

On July 01, 2004 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared BETTY C. LI

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

Notary Seal

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) March 24, 2004
Application No.: (if applicable) 10/807,449

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Rodney Prell	July 1, 2004	, 2004

State of CALIFORNIA }S.S.
County of SAN MATEO }

On July 01, 2004 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared RODNEY A. PRELL

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that he executed the same in his authorized capacity and that by his signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

Notary Seal

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) March 24, 2004
Application No.: (if applicable) 10/807,449

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Sandra Aung	07/01, 2004	, 2004

State of CALIFORNIA }S.S.
County of SAN MATEO }

On July 07, 2004 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared SANDRA AUNG

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

Notary Seal

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Notary

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) March 24, 2004
Application No.: (if applicable) 10/807,449

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Marina Moskalenko	7/01, 2004	, 2004

State of CALIFORNIA }S.S.
County of SAN MATEO }

On July 01, 2004 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared MARINA B. MOSKALENKO

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

ANDREW P. SALVADOR
COMM. # 1349346
NOTARY PUBLIC-CALIFORNIA
SAN MATEO COUNTY
COMM. EXP. APRIL 29, 2006

Andrew P. Salvador
Notary

Notary Seal

Title of Document: ASSIGNMENT

Re:
Title: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS
Filed: (if applicable) March 24, 2004
Application No.: (if applicable) 10/807,449

Name and Signature	Date of Signature	Date Declaration Executed By This Person
Thomas Du	7/1/, 2004	, 2004

State of CALIFORNIA }S.S.
County of SAN MATEO }

On July 01, 2004 before me, ANDREW P. SALVADOR, notary public for and in the State of California, personally appeared THOMAS B. DU,

proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that he executed the same in his authorized capacity and that by his signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

WITNESS my hand and official seal.

Notary Seal

MGC1 ANDREW P. SALVADOR COMM. # 1349346 NOTARY PUBLIC-CALIFORNIA SAN MATEO COUNTY COMM. EXP. APRIL 29, 2006 MGC1

Andrew P. Salvador
Notary

Applicants: Karin Jooss et al.
Application No.: 10/807,449
Express Mail Label No. EM015927105US

Attorney Docket No.: 105576-0033-102
Confirmation No.: 3354

Exhibit D (2 pages)

3802-031-27

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

MARCH 26, 2004

PTAS

102542203A

PIPER RUDNICK LLP
PERRY E. VAN OVER
1200 NINETEENTH STREET, N.W.
WASHINGTON, D.C. 20036-2412

PIPER RUDNICK LLP
MAR 3 0 2004

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 09/02/2003

REEL/FRAME: 014451/0187
NUMBER OF PAGES: 8

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
MOSKALENKO, MARINA — DOC DATE: 08/08/2003

ASSIGNOR:
LI, BETTY — DOC DATE: 08/08/2003

ASSIGNOR:
AUNG, SANDRA — DOC DATE: 08/08/2003

ASSIGNOR:
PRELL, RODNEY — DOC DATE: 08/08/2003

ASSIGNOR:
CRESON, JENNIFER — DOC DATE: 08/19/2003

ASSIGNOR:
JOOSS, KARIN — DOC DATE: 08/08/2003

ASSIGNEE:
CELL GENESYS, INC.
500 FORBES BLVD.
SOUTH SAN FRANCISCO, CALIFORNIA

94080

SERIAL NUMBER: 10404662
PATENT NUMBER:

FILING DATE: 04/02/2003
ISSUE DATE:

MARCUS KIRK, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

Applicants: Karin Jooss et al.
Application No.: 10/807,449
Express Mail Label No. EM015927105US

Attorney Docket No.: 105576-0033-102
Confirmation No.: 3354

Exhibit E (2 pages)

3802-090-27CIP

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

MARCH 10, 2005

PTAS

102831404A

PIPER RUDNICK LLP
PING WANG
SUPERVISOR, PATENT PROSECUTION SERVICES
1200 NINETEENTH STREET, N.W.
WASHINGTON, D.C. 20036-2412

DLA PIPER RUDNICK
GRAY CARY US LLP

MAR 15 2005

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 09/02/2004

REEL/FRAME: 015754/0515
NUMBER OF PAGES: 9

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
JOOSS, KARIN — DOC DATE: 07/01/2004

ASSIGNOR:
CRESON, JENNIFER — DOC DATE: 07/01/2004

ASSIGNOR:
LI, BETTY — DOC DATE: 07/01/2004

ASSIGNOR:
PRELL, RODNEY — DOC DATE: 07/01/2004

ASSIGNOR:
AUNG, SANDRA — DOC DATE: 07/01/2004

ASSIGNOR:
MOSKALENKO, MARINA — DOC DATE: 07/01/2004

ASSIGNOR:
DU, THOMAS

DOC DATE: 07/01/2004

ASSIGNEE:
CELL GENESYS, INC.
500 FORBES BLVD.
SOUTH SAN FRANCISCO, CALIFORNIA

94080

SERIAL NUMBER: 10807449
PATENT NUMBER:
TITLE: CYTOKINE-EXPRESSING CELLULAR VACCINE COMBINATIONS

FILING DATE: 03/24/2004
ISSUE DATE:

ALLYSON PURNELL, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS